The Beginning of the Ends: Circularizing Linear Chromosomes in *Saccharomyces cerevisiae*

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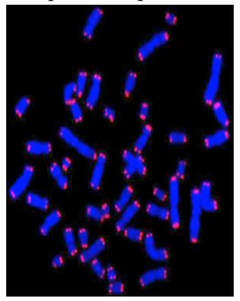
Abstract

Both circular and linear chromosomes exist in nature. Generally, prokaryotes contain a single circular chromosome while eukaryotes contain multiple linear chromosomes. However, the termini of linear chromosomes cannot be fully replicated. These ends of linear chromosomes are called telomeres, which are composed of repetitive DNA sequences that "cap" and protect DNA ends. To combat the end-replication problem at telomeres, most eukaryotes require the enzyme telomerase. Telomerase synthesizes DNA at the telomere to prevent shortening during been replication. On the other hand, the circular chromosomes of prokaryotes have no telomeres, need no telomerase, and do not shorten over time. This leads one to wonder: why did linear chromosomes evolve if they are unable to replicate their ends and require the presence of telomerase? In this project, we are genetically modifying the simple eukaryote *Saccharomyces cerevisiae* to convert each of their linear chromosomes into circular chromosomes. I will insert a DNA cassette into the ends of chromosome I and select for a recombination event that circularizes the linear DNA. Once completed, the viability and health of the modified chromosomes will be assessed. This may give us insights as to why linear chromosomes evolved.

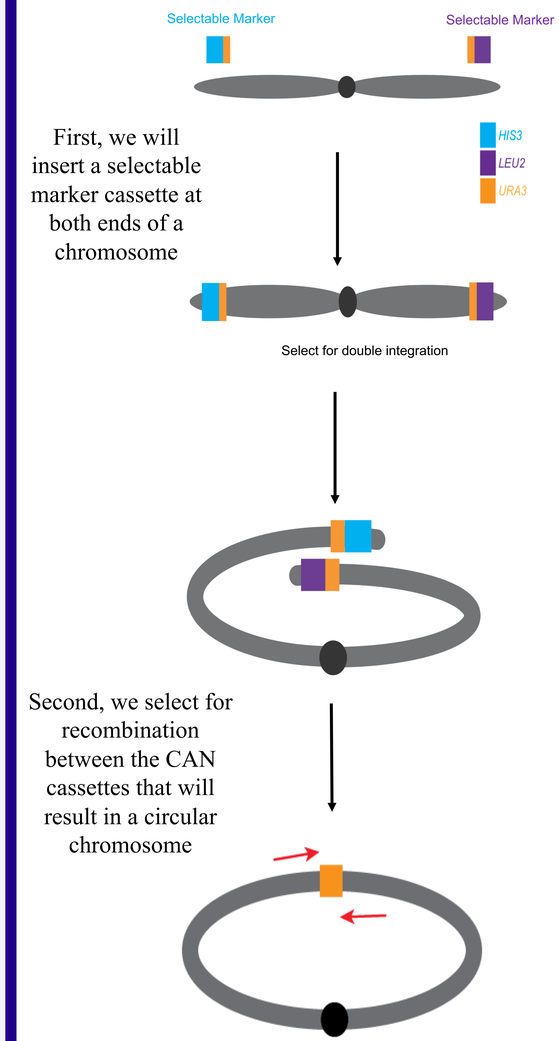
Background

Telomeres are repetitive DNA sequences at the end of eukaryotic chromosome. The DNA replication machinery cannot fully copy telomeres, leading to an end-replication problem.



This figure shows a chromosome spread with DNA dyed a teal color and the telomeric region dyed pink.

Experimental Approach

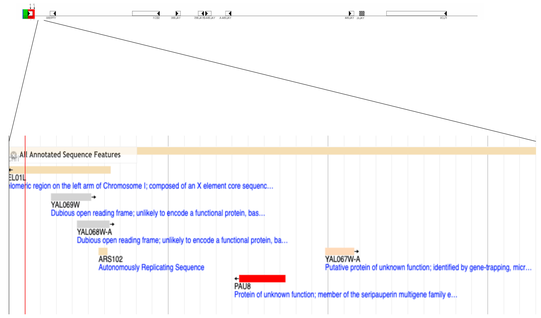


To verify circularization, we will perform PCR with primers (red arrows) across the junction.

Details on Chromosome I

I chose chromosome I because it is the shortest chromosome in *Saccharomyces cerevisiae* cells. The right side of the chromosome has a DNA sequence that was completely unique from the rest of the genome, so there is a strong chance of the cassette being inserted in the right area. On the other hand, the left side of the chromosome shows similarities to multiple sections of seven other chromosomes (a near 100% match with chromosome X). With that, the insertion will have to be more carefully done to ensure it in the correct spot.

Left Arm of Chromosome I



Right Arm of Chromosome I

Materials and Methods

***Saccharomyces cerevisiae* (baker's yeast)**
For our research, a model organism. *Saccharomyces cerevisiae* is a simple eukaryotic organism with 16 chromosomes. It is able to easily manipulated and has a well annotated genome

Polymerase Chain Reaction (PCR)
This is a method widely used in molecular biology to make many copies of a certain segment of DNA. Developed in Kary Mullins in 1982, the PCR process involves the heating and cooling of DNA with polymerase to amplify and generate thousands of copies of a small, particular segment of DNA.

DNA (Gene) Cassette
Once a gene and a recombination site are added to a strand of DNA (through PCR or other methods), the modular piece of DNA is deemed a "DNA cassette". Our DNA cassettes will contain: marker genes and homology to the telomeric region

Current Progress

This semester, we have analyzed the telomeric region specific to our chromosome and designed the PCR primers that will build our DNA cassettes.

Future Goals

We hope we are able to circularize all 16 chromosomes and assess their health. Based on previous research and projects carried out in the past, this should be able to be accomplished. The broader object of this project is to determine why linear chromosomes may have evolved from circular chromosomes many years ago.

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